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Mechanisms Involved in Uptake of *Bordetella bronchiseptica* by Mouse Dendritic Cells

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The invasion and intracellular survival of *Bordetella bronchiseptica* in mouse dendritic cells were investigated. The results obtained suggest that *B. bronchiseptica* binds specifically to glycosylated receptors present on the plasma membrane of dendritic cells, thereby inducing a signal that triggers an actin polymerization-dependent phagocytic process, probably via a protein kinase-dependent transducing phosphorylation signal. The energy required for the uptake process by host cells is provided mainly by the glycolytic pathway. An intact microtubule system and de novo protein synthesis in eukaryotic and prokaryotic cells are essential for efficient uptake and intracellular survival. The interaction of *B. bronchiseptica* with dendritic cells may be pertinent to natural infections that follow a chronic clinical course and predispose to secondary infections, and to the T-cell response involved in protective immunity following infections caused by *Bordetella* spp.

Bordetella bronchiseptica is associated with different pathological syndromes characterized mainly by a mild and chronic clinical course in several animal species (21, 34, 59). Traditionally, the toxins and adhesins produced by these microorganisms were considered the main factors involved in pathogenesis of *Bordetella* spp. (3, 21, 23, 27, 34, 47, 59), although an increasing body of evidence supports a general invasive capability for *B. bronchiseptica* (50, 51) and other *Bordetella* spp. (5, 8, 11, 13, 17, 33, 46, 49). Particularly significant in the pathogenesis of *Bordetella pertussis* appear to be invasion and intracellular survival in macrophages (8, 17, 49).

We have recently reported that *B. bronchiseptica* is efficiently internalized by mouse dendritic cells (DC) and survives intracellularly for at least 72 h (25). The interaction with DC could be relevant in the infective processes caused by *B. bronchiseptica* since DC are present in the airways (38, 53), the portal of entry for *B. bronchiseptica*, and are the most efficient antigen-presenting cells (57, 58). This interaction might explain, at least in part, the chronic clinical course of the disease, the secondary infections that commonly occur, and the type of immune response elicited during natural infection. In the present study, a fully functional murine spleen DC line was used to characterize the mechanisms of bacterial uptake and persistent intracellular survival.

MATERIALS AND METHODS

Bacterial strains and media. The wild-type *B. bronchiseptica* 5376 was used throughout this work (60) and cultivated at 37°C on Bordet-Gengou agar base (Difco Laboratories, Detroit, Mich.) supplemented with 1% glycerol and 15% (vol/vol) defibrinated horse blood, in SS broth (54), and in brain heart infusion broth or agar (Difco Laboratories). Liquid cultures were aerated by shaking at 300 rpm in a New Brunswick Environmental Incubator Shaker.

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Tissue culture methods and invasion assays. The murine spleen DC line CB1 obtained from DBA/2 mice (40) was maintained in Iscove's modified Dulbecco's medium (Sigma Chemie GmbH, Deisenhofen, Germany) supplemented with 5% fetal calf serum and 5 mM glutamine (GIBCO Laboratories, Eggenstein, Germany) in an atmosphere containing 5% CO_2 at 37°C. Cells were seeded at a concentration of approximately 5×10^4 per well in 24-well Nunclon Delta tissue culture plates (Inter Med NUNC, Roskilde, Denmark), incubated for 18 h, and washed twice with complete medium. *B. bronchiseptica* was grown for infection assays on Bordet-Gengou agar for 24 h, recovered with sterile swabs, and suspended in complete Iscove's medium, and the suspensions were adjusted spectrophotometrically to an optical density at 540 nm corresponding approximately to 2×10^7 CFU ml^{-1}. Then 0.5 ml of each of the suspensions was added to a well of the DC-containing tissue culture plates, and the plates were incubated statically for 2 h. Supernatant fluids were subsequently discarded, and the cells were washed twice with phosphate-buffered saline (PBS; NaCl [8.0 g liter^{-1}], KCl [2.0 g liter^{-1}], $Na_2HPO_4 \cdot 2H_2O$ [2.0 g liter^{-1}], KH_2PO_4 [2.0 g liter^{-1}] [pH 7.4]) to remove nonadherent bacteria. The medium was replaced with 0.5 ml of complete Iscove's medium supplemented with 100 μg of gentamicin (Sigma Chemie GmbH) ml^{-1}, and the mixture was incubated at 37°C for 2 h to kill remaining extracellular bacteria. The supernatant fluids were discarded, and the cells were washed twice with PBS to remove residual gentamicin. The cells were then lysed by addition of 0.5 ml of water to each well, and the number of CFU recovered from each well was determined by plating 10-fold dilutions on Bordet-Gengou or brain heart infusion agar with a Spiral Plater model C (Spiral Biotech, Inc., Bethesda, Md.). The results reported are mean values of three independent experiments ± the standard error of the mean. Incubation of *B. bronchiseptica* bacteria suspended to a density equal to that used in the invasion assays in Iscove's medium supplemented with gentamicin at 100 μg ml^{-1} for 2 h resulted in a greater than 6 orders of magnitude reduction in CFU.

Pretreatment of DC or bacteria with inhibitors, activators, or blocking compounds. In several experiments, DC or bacteria were pretreated with monodansylcadaverine (MDC; 50

μM), cytochalasin D (CD; 0.5 μg ml^{-1}), nocodazole (3 μg ml^{-1}), mitomycin (40 μg ml^{-1}), sodium fluoride (NaF; 20 mM), iodoacetic acid (0.2 mM), 2,4-dinitrophenol (2 mM), heparin (100 U ml^{-1}), DEAE dextran (DEAED; 30 μg ml^{-1}), tunicamycin (1 μg ml^{-1}), neuraminidase type V from *Clostridium perfringens* (0.1 U ml^{-1}), actinomycin D (AD; 1 μg ml^{-1}), chloramphenicol (50 μg ml^{-1}), staurosporine (1 μM), genistein (200 μM), calphostin C (0.35 μM), phorbol 12-myristate 13-acetate (PMA; 4 μM), NH_4Cl (10 mM), chloroquine (10 μg ml^{-1}), the lipid-soluble cyclic AMP (cAMP) derivative N^6,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate monosodium salt (cAMPd; 2 mM), the calcium ionophore A23187 (1 μM), concanavalin A (1 μg ml^{-1}), recombinant tumor necrosis factor (Genzyme Corp., Cambridge, Mass.; 100 U ml^{-1}), lipopolysaccharide from *Escherichia coli* O26:B6 (0.5 ng ml^{-1}), recombinant murine gamma interferon (IFN-γ) (Genzyme Corp.; 20 U ml^{-1}), granulocyte-macrophage colony-stimulating factor (GM-CSF) (Genzyme Corp.; 500 ml^{-1}), a synthetic peptide containing the RGD sequence present in filamentous hemagglutinin (FHA; GNVTVGRGD PHQGVL, RGD-pep; 10 μg ml^{-1}), a control peptide containing FHA sequences located downstream of the RGD region (PHQGVLAQGDIIMDA, cont-pep; 10 μg ml^{-1}), galactose (50 mM), and rabbit polyclonal antibodies raised against the FHA or the outer membrane protein pertactin (1:100 dilution).

Inhibition experiments were also performed with the following antibodies specific for surface proteins and receptors of DC, together with negative-control antibodies specific for markers absent in DC: monoclonal antibody (MAb) against Thy-1 (clone ATCC HB23), MAb against FA11 (from R. M. Steinman), MAb against MAC-2 (clone ATCC TIB166), MAb against CD11a (clone ATCC TIB213), MAb against FcγRII (clone ATCC HB197), MAb against F4/80 (clone ATCC HB198), MAb against CD11b (clone ATCC TIB128), MAb against ICAM-1 (clone ATCC CRL1878), and MAb against CD11c (clone ATCC HB224).

Cells were pretreated for 1 h before addition of bacteria and throughout the invasion assays, except when the following compounds were used: PMA that was used for short-term (30-min) and long-term (24-h) pretreatments; IFN-γ, GM-CSF, and lipopolysaccharide (24 h before and throughout the experiment); and DEAED (two washes before infection). The hybridoma supernatants containing MAb specific for surface proteins were used at a 1:4 dilution to pretreat DC for 2 h. All compounds for which there is no specific information were provided by Sigma Chemie GmbH. The concentration of the inhibitor used was selected on the basis of data obtained in preliminary experiments, using values reported in the literature for several cell systems (2, 11, 12, 17, 19, 24, 26, 29, 32, 33, 39, 41, 48, 62); when possible the activity of the inhibitor was established by using control bacteria (e.g., inhibition by CD of *Listeria monocytogenes* uptake) or assessing the biological activity (e.g., pH of the intracellular compartment by using acridine orange after pretreatment with chloroquine or NH_4Cl; staining of the microtubules after pretreatment with nocodazol). Treatments of bacteria and DC with the different compounds did not significantly reduce cell viability (data not shown).

Scanning electron microscopy. Infected cells on round 13-mm Thermanox coverslips (Inter Med NUNC) were fixed in a PBS solution containing 3% glutaraldehyde and 5% formaldehyde for 45 min on ice, washed with PBS, dehydrated in a graded series of acetone concentrations, and subjected to critical-point drying with CO_2. The samples were then covered with a 10-nm gold film and examined with a Zeiss DSM 940 scanning electron microscope.

Statistical calculations. The results obtained after pretreatment with inhibitors were analyzed for significance by analysis of variance and by Student's *t* test. Differences were considered significant at $P \leq 0.05$.

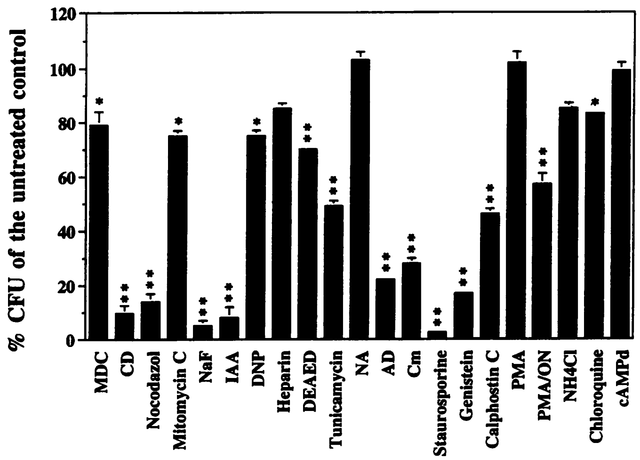

FIG. 1. Influence of various bioactive reagents on the uptake of *B. bronchiseptica* bacteria by mouse dendritic cells. Dendritic cells were pretreated with the indicated substances as described in Materials and Methods prior to addition of *B. bronchiseptica* bacteria. Results are expressed as a percentage of the CFU recovered per well from the untreated control (mean, $2.5 \times 10^6 \pm 6 \times 10^4$) and represent mean values of three independent experiments ± standard errors of the mean. The results are statistically significant when compared with the untreated controls at $P \leq 0.05$ (*) and $P \leq 0.001$ (**). Abbreviations: IAA, iodoacetic acid; DNP, 2,4-dinitrophenol; NA, neuraminidase type V; Cm, chloramphenicol.

RESULTS AND DISCUSSION

Mechanisms involved in the uptake of *B. bronchiseptica* by DC: microfilament-dependent versus receptor-mediated endocytosis. Macromolecules and small particles generally enter phagocytic and nonphagocytic cells by one of two processes: microfilament-dependent phagocytosis or receptor-mediated endocytosis in which receptor-bound particles are clustered in specialized membrane regions and rapidly invaginated (20). To assess which process was involved in the uptake of *B. bronchiseptica*, cells were pretreated with the depressor of receptor-mediated endocytosis MDC (52) or the inhibitor of actin polymerization CD. The very weak inhibitory effect observed following pretreatment with MDC, and the 90% reduction in the number of viable bacteria observed after pretreatment with CD, suggested that *B. bronchiseptica* is taken up by DC through microfilament-dependent phagocytosis (Fig. 1). We cannot exclude the possibility that the strong effect of CD is due in part to the major morphologic alterations also produced, which might reduce bacterial attachment (Fig. 2C and D), although bacteria seemed to attach efficiently to CD-treated cells (Fig. 2C and D). Moreover, the shape of DC pretreated with MDC was also affected to a similar extent, without a significant reduction in the number of viable microorganisms (data not shown). Experiments involving short-term bacterial binding to untreated and pretreated DC were performed at 4°C to confirm that pretreatments do not affect attachment. The results obtained confirmed that bacterial binding was not significantly impaired by the use of inhibitors, because the

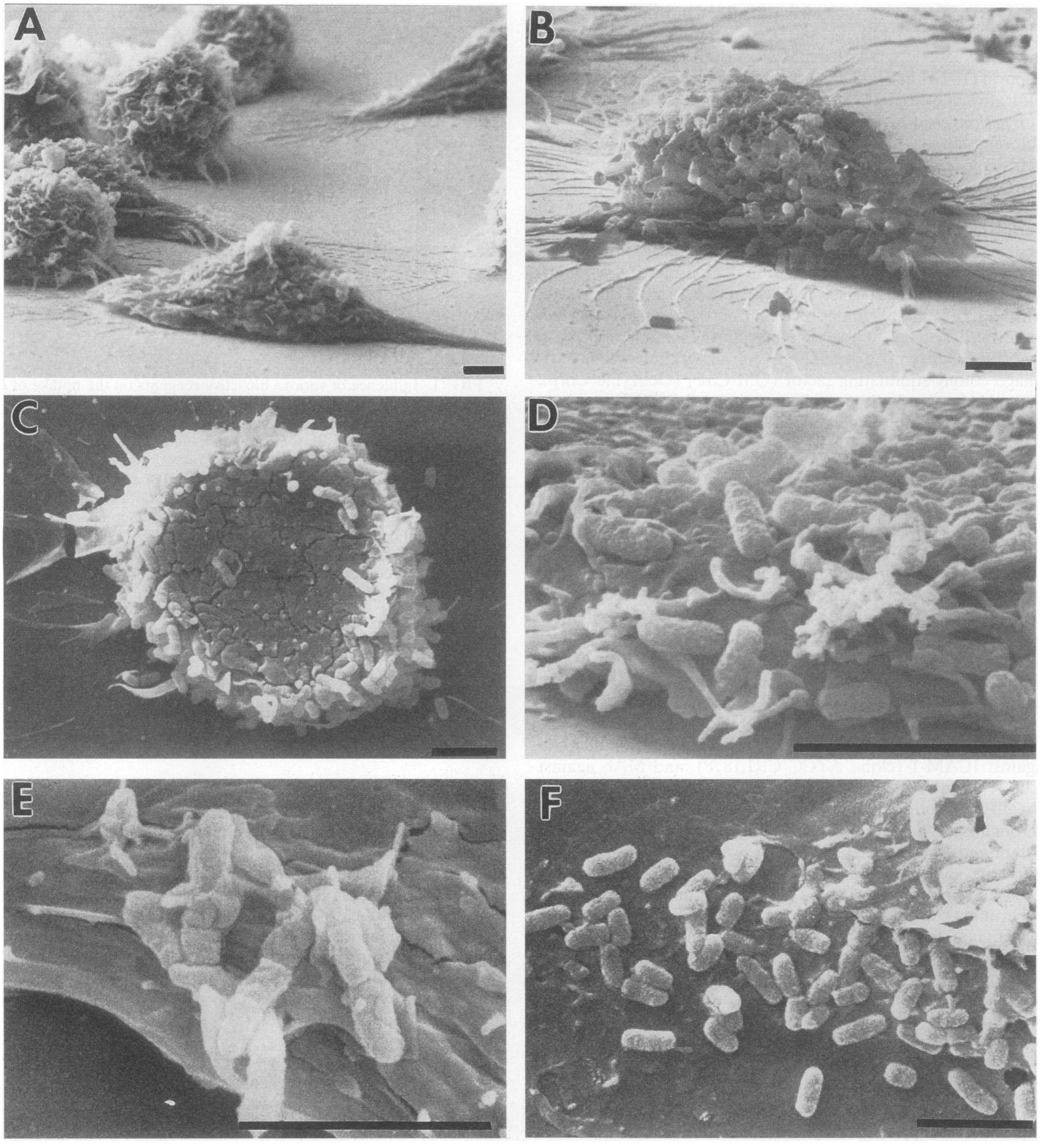

FIG. 2. Scanning electron microscopy analysis of dendritic cells infected by *B. bronchiseptica*. (A) Uninfected cells; (B) untreated cells infected with *B. bronchiseptica* 5376; (C and D) infected cells pretreated with CD; (E) cells infected with bacteria pretreated with polyclonal antibodies against outer membrane protein; (F) infected cells pretreated with MAb against CD11c. Panels C, E, and F are top views, whereas panels A, B, and D are tilted views of the sample (75°). Bars, 2.5 μm.

difference in the mean number of bacteria recovered from untreated and pretreated DC after 30 min and 1 and 2 h of interaction at 4°C was not statistically significant ($P \geq 0.05$). Bacteria preferentially attached to the cell periphery, as can best be seen in CD-treated cells with a flat shape (Fig. 2C and D).

Role of the microtubule system. The movement of cytoplasmic organelles is mediated by both microfilaments and microtubules (1, 36), which are also central structural components of the cell framework. The role of the microtubule system in the uptake process was analyzed by inhibiting microtubule polymerization with nocodazole: this resulted in an 86% inhibition in the uptake of *B. bronchiseptica* (Fig. 1). This effect may be due to the very complex microtubule network in DC, which may be essential for their highly specialized functions, or to microtubule involvement in some step(s) of the microfilament-dependent phagocytosis. Alternatively, *B. bronchiseptica* may be taken up by a second microtubule-dependent endocytic pathway. This possibility is not unlikely, given that a similar mechanism, which coexists with the classical microfilament-dependent pathway, has been recently demonstrated for some strains of *Campylobacter jejuni* and *Citrobacter freundii* (39). Moreover, an intact microtubule network was reported to be essential for maintenance of *Salmonella*-induced filaments which are correlated with intracellular bacterial replication (19).

To assess whether the effect of nocadozole might be due in part to inhibition of the mitotic process, which also involves microtubules, cells were pretreated with mitomycin, an inhibitor of DNA synthesis and nuclear division. The slightly smaller number of viable microorganisms recovered suggested that the effect of blocking cell division is very weak or insignificant (Fig. 1).

Source of energy for the uptake process. Endocytic processes require energy that is generated in host cells by glycolytic or oxidative metabolic pathways (26). To assess the contribution of these two potential pathways, cells were pretreated with iodoacetic acid or NaF, which block the glycolytic route, or with 2,4-dinitrophenol, which impairs the oxidative pathway. The 95, 92, and 25% reduction in the number of CFU observed after pretreatment with iodoacetic acid, NaF, and 2,4-dinitrophenol, respectively, suggests that DC derive the energy for uptake of *B. bronchiseptica* mainly from glycolysis.

Specific versus nonspecific binding. It has been reported that polyanions or polycations may affect infection rates of eukaryotic cells by modifying the cytoplasmic membrane and impairing nonspecific binding (32). To study the role of nonspecific electrostatic interactions in the attachment of *B. bronchiseptica* to DC, cells were treated with heparin and DEAED. The 15 and 30% inhibition in uptake observed after heparin and DEAED pretreatment, respectively (Fig. 1), suggests that electrostatic interactions play a minor role in the bacterial attachment that leads to invasion and that binding to a specific receptor(s) is the dominant mechanism involved.

DC constitutively express on their surface high levels of adhesion and costimulatory molecules implicated in cell-cell adhesion and signaling (57). Many of these receptors (e.g., β-integrins) are glycosylated proteins which are candidates as receptors for specific ligands. To analyze the potential role of glycosylated proteins as receptors for *B. bronchiseptica*, DC were treated with tunicamycin, an inhibitor of N-glycosylation. The 51% inhibition achieved (Fig. 1) suggests that glycosylated surface proteins may mediate a specific binding process that precedes the invasion process. Although *B. bronchiseptica* attaches to sialyl glycoconjugates present in swine nasal mucosa (29), treatment of DC with neuraminidase type V did not reduce bacterial uptake, which suggests that receptors containing sialyl moieties may not be involved in the internalization process.

Role of de novo protein synthesis. The requirement of energy and the likelihood of receptor-adhesin systems in the uptake process prompted us to analyze the role of de novo protein synthesis. DC treated with AD, an inhibitor of eukaryotic cell protein synthesis, and bacteria treated with chloramphenicol, an inhibitor of prokaryotic protein synthesis, exhibited 78 and 72% reductions in uptake of bacteria, respectively (Fig. 1). This suggested that de novo synthesis in both DC and *B. bronchiseptica* may be necessary for optimal uptake and that efficient intracellular survival of bacteria may also require the synthesis of new bacterial proteins. A similarly strong dependence of invasion upon bacterial de novo protein synthesis was demonstrated for *Campylobacter jejuni* and *Citrobacter freundii*, whereas no such dependence was demonstrated for invasion by *Salmonella* spp. and *Yersinia enterocolitica* (16, 35, 39).

Transduction signals involved in the uptake process. The results reported above suggest that attachment of *B. bronchiseptica* to DC surface receptors generates an uptake signal that triggers a microfilament-dependent internalization mechanism. Protein kinases (PK) are often involved in the transduction of signals generated by surface receptors (42), using phosphorylation to activate other proteins that may in turn effect internalization, as has been described for other invasive microorganisms (18, 48). PK are also involved in the regulation of surface receptors (e.g., integrins), adhesion, and cytoskeletal reorganization (30, 42). To assess whether PK are involved in the uptake process, DC were treated with the wide-range PK inhibitor staurosporine, the protein kinase C (PKC) inhibitor calphostin C, and the tyrosine PK inhibitor genistein. The results obtained indicated that more than one PK is involved in the transduction mechanism or intracellular survival. The most severe reduction (83%) was obtained with inhibitors of tyrosine PK, but a significant reduction (54%) was also seen with the PKC inhibitor.

PMA has been shown to increase PKC levels during short-term treatments but to lower them to calphostin C treatment levels during longer-term (overnight) treatments (12). To further assess the role of PKC in the uptake process, DC were treated overnight with PMA; the intracellular survival of bacteria was reduced to an extent (43%) similar to that of calphostin C-treated cells (54%). This supports a role of PKC in the internalization process. Other invasive bacteria are readily phagocytized by cells pretreated with PK inhibitors (48), so the inhibitory effect seems to be specific for *B. bronchiseptica* (and perhaps DC) and not to be a general effect on phagocytosis. The activity of PK might result in part from effects on the microtubule system (see above), since microtubules are critically regulated by proteins whose activities are controlled by phosphorylation (24).

Intracellular survival of *B. bronchiseptica*. After internalization, *B. bronchiseptica* is able to survive intracellularly for at least 72 h (25). In principle, invasive bacteria that do not escape from the phagosome can survive by several different mechanisms: inhibition of the respiratory burst, resistance to lysosomal enzymes, attenuation of phagosome acidification, inhibition of phagolysosome fusion, and/or targeting following endocytosis to an unfused compartment (2, 14–16). The first two mechanisms seem unlikely, since damaged bacteria were present within phagolysosomes 4 h after infection (25) and it has been previously demonstrated that the respiratory burst occurs at normal levels in polymorphonuclear cells infected with *Bordetella* spp. (55, 56).

Ammonium chloride has been shown to inhibit the phagolysosome fusion process and to impair endosome acidification (22), whereas chloroquine raises the pH of the intracellular compartment, thereby affecting proteolytic activity (4, 63). Therefore, if the phagolysosome fusion or the phagosome acidification processes are unaffected by *B. bronchiseptica*, these compounds may improve bacterial survival. Conversely, acidification of the endocytic compartment might be necessary for bacterial survival, as has been demonstrated for *Salmonella* spp. and *Coxiella burnetii* (14, 19). However, these compounds had no significant effect on bacterial survival (Fig. 1), suggesting that *B. bronchiseptica* may impair the phagolysosome fusion and/or acidification and that this inhibition cannot be in-

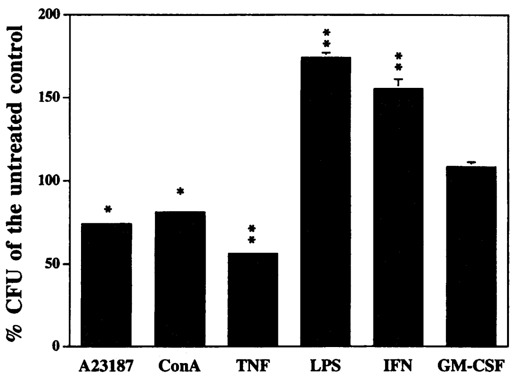

FIG. 3. Effect of activation of dendritic cells on the uptake and intracellular survival of *B. bronchiseptica*. Results are expressed as a percentage of the CFU recovered per well from the untreated control (mean, $2.5 \times 10^6 \pm 6 \times 10^4$) and represent mean values of three independent experiments ± standard errors of the mean. Results are statistically significant when compared with the untreated controls at $P \leq 0.05$ (*) and $P \leq 0.001$ (**). Abbreviations: ConA, concanavalin A; TNF, tumor necrosis factor; LPS, lipopolysaccharide.

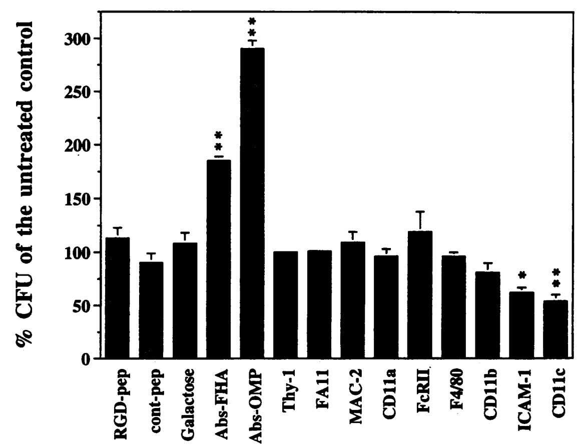

FIG. 4. Effect of adhesin and receptor inhibitors on the uptake of *B. bronchiseptica* by mouse dendritic cells. Results are expressed as a percentage of the CFU recovered per well from the untreated control (mean, $2.5 \times 10^6 \pm 6 \times 10^4$) and represent mean values of three independent experiments ± standard errors of the mean. Results are statistically significant when compared with the untreated controls at $P \leq 0.05$ (*) and $P \leq 0.001$ (**).

creased by other compounds. Such mechanisms for survival in the vacuolar nonpermissive environment have been proposed for *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Toxoplasma gondii*, and *Salmonella* spp. (6, 14, 28). Alternatively, *B. bronchiseptica* might be targeted to an unfused compartment, as has been demonstrated for *M. tuberculosis* (37).

Short-term PMA treatment has been reported to impair the infectivity of intracellular pathogens by improvement of the metabolic burst, superoxide production, and phagolysosome fusion (12). cAMP analogs also suppress infection of eukaryotic cells by invasive bacteria by mimicking the effect of high cAMP levels that favors the release of lysosomic enzymes inside phagosomes (9, 61). The lack of effect after short-term pretreatment with PMA and cAMPd supports the hypothesis that *B. bronchiseptica* is targeted to a unfused compartment and is in agreement with electron microscopy results (25).

Effect of DC activation. Nonspecific cell activators and stimulating factors, like ionophores, concanavalin A, and lipopolysaccharide impair the infectivity of invasive microorganisms and result in the production of cytokines, such as tumor necrosis factor, IFN-γ, and GM-CSF (7, 10, 31, 41, 45), involved in the inflammatory response to infectious microorganisms. These activators also enhance DC viability and/or function (57), inducing the production of surface molecules and improving maturation. The anti-infective effect of these compounds could be due to an increase in the metabolic burst, phagolysosome fusion, phagocytosis, PKC, Ca^+ influx, or superoxide generation or to downregulation of transferrin receptor (43). This prompted us to analyze the effect of DC activation by different compounds on bacterial intracellular survival. The results given in Fig. 3 show that while A23187, concanavalin A, and, particularly, tumor necrosis factor exhibited antibacterial activity (26, 19, and 44% inhibition, respectively), lipopolysaccharide and IFN-γ increased the number of viable intracellular bacteria (174 and 155% of the control). This suggested that either the production of new receptor molecules was induced (e.g., IFN-γ induces posttranslational modifications) or DC became more active in endocytosis. GM-CSF displayed very little effect, if any at all (108% of the control).

Adhesins and receptors. FHA mediates adhesion to and invasion of macrophages by *Bordetella* spp. through interaction with the integrin complement receptor 3 via its RGD-containing domain and adhesion to other cells via a galactose-binding domain (33, 46, 49). Therefore, the possible role of this adhesin in the interaction with DC was analyzed. No inhibition was obtained when RGD-pep and galactose were used (113 and 108% of the control, respectively), suggesting that neither the RGD sequence nor the galactose-binding domains of FHA are involved in the invasion of DC (Fig. 4). A polyclonal antibody against FHA also failed to inhibit invasion, which tends to exclude other potential binding sites in FHA (Fig. 4). These results are in agreement with the uptake and intracellular survival of the *B. bronchiseptica bvg* mutant that lacks FHA (25).

The internalization of *B. pertussis* by mammalian cells might also be mediated by the RGD sequence present in outer membrane protein 69 (33). However, pretreatment with antibodies against outer membrane protein did not impair uptake but resulted in an enhancement, as was seen with antibodies against FHA (Fig. 3). This increase in the uptake could be due to the presence of agglutinating antibodies, which result in the formation of clumps that may be endocytosed more easily (Fig. 2G). Alternatively, opsonized bacteria might bind to the few FcγRII receptors present in DC that could in turn have a minor role in either binding or internalization.

To identify the cellular receptors involved in the specific binding that leads to internalization, antibodies that recognize surface proteins synthesized by DC were used in inhibition experiments (Fig. 4). Pretreatment with MAbs against the surface receptors MAC-2, CD11a, FcγRII, and F4/80, which are expressed at low or intermediate levels by DC (40, 57), had no effect on internalization (109, 96, 119, and 96% of the control, respectively); MAb against CD11b that is expressed at intermediate levels produced only 19% inhibition; whereas MAbs against ICAM-1 and CD11c that are expressed at high levels resulted in 38 and 46% inhibition, respectively (Fig. 4). This could be explained either by the specific blocking of the receptor involved in the internalization or by a nonspecific masking of unknown surface structures implicated in the binding that leads to the uptake process. However, attachment rates were unaffected by treatment with MAbs against ICAM-1 and CD11c (Fig. 2F). MAbs against Thy-1 (expressed only by T lymphocytes) and FA11 (DC endocytic vacuole component) were used as control antibodies and had no effect (Fig. 4).

Taken together, these results lead us to hypothesize that *B. bronchiseptica* bacteria bind specifically to membrane glycosylated receptors of DC, providing a signal that triggers an actin polymerization-dependent endocytic process, probably via a PK-dependent transducing phosphorylation signal. The energy required for the uptake process is provided by the glycolytic pathway of host cells. An intact microtubule system and de novo protein synthesis in eukaryotic and prokaryotic cells are also essential for optimal uptake and intracellular survival. On the one hand, the presence of *B. bronchiseptica* within intact DC may favor chronicity and could lead to an impaired immune response. The alterations in the immune response resulting from DC infection may explain the characteristic clinical course and the secondary infections that often complicate syndromes caused by this pathogen. On the other hand, persistent infection of DC could favor major histocompatibility complex class I-restricted antigen presentation, leading to a strong T-cell response, which seems essential in the protective immunity acquired during natural infections (44).

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